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Evaluation of Possible Human Health Risk of Heavy Metals from the Consumption of Two Marine Fish Species *Tenualosa ilisha* and *Dorosoma cepedianum*

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Abstract

In order to study the effects of contaminants on human health, fish is considered as a powerful model among all available species for risk-benefit assessments. *Tenualosa ilisha* and *Dorosoma cepedianum* are two fish species of great economic importance as they are found in undeveloped, developing and developed countries. Concentrations of heavy metals lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As) and mercury (Hg) were determined using validated and accredited test methods in order to assess the potential human health risk from the dietary intake of these two selected fish species. The estimated daily intake (EDI) of all the five heavy metals was measured from the consumption of the two species considering the mean fish consumption of 61 g person^{-1} day^{-1} defined for European population. The EDI indicates that no risk to people's health with respect to the EDI of Pb, Cd, Cr, As and Hg through the consumption of the two fish species. The estimation of target hazard quotient (THQ) demonstrating the non-carcinogenic risk indicates that intake of Pb, Cd, Cr and Hg through the consumption of two fish species is safe for human health, whereas, consumption of As suggests potential risk to consumers. The estimation of carcinogenic risk of Cd, Cr and As due to the consumption of two selected fish species indicates that consumers remain at risk of cancer. Thus, these fish species should not be considered safe for human consumption.

Keywords Heavy metals · Marine fish · *Tenualosa ilisha* · *Dorosoma cepedianum* · Human health risk · Carcinogenic and non-carcinogenic risk

Introduction

Fish is highly nutritious due to its high contents of protein, essential fats, vitamins and minerals [1]. It contributes about 17% of animal protein consumed by the world population [2]. But, the aquatic environment is becoming polluted due to various reasons that include industrial effluent discharge, agricultural discharge, sewage drainage, gasoline from fishing boats and accidental chemical waste spills [3, 4]. Thus, a wide variety of chemical contaminants including heavy metals are continuously entering into the aquatic ecosystem where they pose severe threat because of their toxicity, stable, non-biodegradable and biomagnification in the food chain [5]. Fish indicates the pollution status of water and hence is considered as an excellent biomarker of heavy metals in aquatic ecosystem [6].

Human beings are potentially exposed to heavy metals through the food chain with consumption of fish. Exposure to heavy metals results in various health effects. For example, exposure to lead disrupts the cognitive development in children and increases blood pressure and cardiovascular disease in adults [7]. Moreover, prolonged exposure to lead results in mental retardation, coma and even death [8]. Exposure to cadmium injures the kidney function and causes chronic toxicity including hypertension, poor reproductive capacity, tumorous and hepatic dysfunction [8, 9]. Moreover, cadmium toxicity may result endocrine disruption, genotoxicity, oxidative damage, disruption of ion regulation [10]. Chromium toxicity may result severe respiratory, gastrointestinal, haematological, hepatic, renal, cardiovascular and neurological effects

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as part of the sequelae leading to death [11]. Exposure to arsenic can affect almost all organ systems including the dermatologic, nervous, renal, hepatobiliary, gastro-intestinal, cardiovascular and respiratory systems [12, 13]. The toxicity of mercury includes gastrointestinal toxicity, nephrotoxicity and neurotoxicity [12, 13]. Therefore, much attention has been paid in recent decades to investigate the level of heavy metals in foodstuffs as a result of growing concern of public health due to food consumption.

The exceedance of the recommended values of heavy metals set by different regulatory bodies does not always represent the human health risk, and consequently, in recent years, the health risk assessment has been extensively used to evaluate the impact of the hazards of heavy metals bioaccumulation on human health. Assessment of potential risk of heavy metals on human health is evaluated through non-carcinogenic and carcinogenic effects [14]. The non-carcinogenic health risk is generally evaluated by the target hazard quotient (THQ) value and/or total target hazard quotient (TTHQ) measurement set by United States Environmental Protection Agency [15]. The carcinogenic risk (CR) due to the lifetime exposure of heavy metals is evaluated by using carcinogenic slope factor also set by United States Environmental Protection Agency [15].

In the present study, levels of five heavy metals lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As) and mercury (Hg) in two marine fish species *Tenualosa ilisha* and *Dorosoma cepedianum* were measured using validated and accredited test methods [16]. *Tenualosa ilisha* is found in the Indian Ocean from the gulf, east to the coasts of India, as far as Myanmar [17]. It is also found in the Tigris River, the Gulf of Tonkin (Viet Nam) and other rivers of southern Iran [18, 19]. The range of *Dorosoma cepedianum* includes Mississippi River, St. Lawrence-Great Lakes, Atlantic and Gulf drainages from Quebec to North Dakota and New Mexico, south to southern Florida and Mexico, introduced outside native range [20]. An assessment on non-carcinogenic and carcinogenic human health risks associated with the dietary intake of heavy metals through the consumption of the two selected fish species has been conducted.

Materials and Methods

Collection and Preservation of Test Samples

The countries in which *Tenualosa ilisha* is found are Bangladesh, India, Pakistan, Myanmar, Thailand, Sri Lanka, Malaysia, Iraq, Iran, Kuwait, Qatar, Oman, United Arab Emirates, Saudi Arabia and Vietnam [17]. *Dorosoma cepedianum* species is found in Canada, Mexico and USA [17]. The distributions of *Tenualosa ilisha* and *Dorosoma cepedianum* are shown in Figs. 1 and 2 respectively. The two fish species are mainly imported to Bangladesh and then distributed from the capital city, Dhaka, throughout the country. Fresh samples of two selected fish species (20 specimens each) were purchased from four different wholesale markets of Dhaka, Bangladesh, namely, Mirpur-1, Mohammadpur Town Hall, Newmarket and Karwan Bazar (Fig. 3). The samples were wrapped in polyethylene bags and transported to the Analytical Chemistry Laboratory, Atomic Energy Centre Dhaka and Bangladesh Atomic Energy Commission. The samples immediately after transportation to the laboratory were washed with fresh water following with deionised water with a view to remove the mud or other fouling substances. Then, the muscle tissue of each sample was removed, chopped into pieces with the aid of a steam cleaned stainless steel knife and homogenised in a food processor. The homogenised muscle tissues were then air-dried to remove the extra water and 200 g of test portions were stored at − 20 °C. Metal contents were expressed as milligram per kilogram wet-weight basis of fresh fish. In order to compare the results obtained in the present study, with the previously published studies, the dry weight data, those were reported as dry weight, are converted to wet weight using a conversion factor 4 [21].

Digestion of Samples

Microwave digestion system has been adopted to perform chemical digestion under extreme temperature and pressure in chemical laboratories in order to prevent the loss of analytes from the matrix. A microwave oven (MARS′5 XP-1500 plus, CEM Corporation, USA) was used in preparation of samples for metal analysis. One gram of each homogenised fish sample was placed into the polytetrafluoroethylene digestion vessel and 6 mL of *suprapur* nitric acid was added to it as a digestion reagent. The maximum power of the rotating magnetron was 1600 W. Two-step temperature program was used for the digestion of the samples. During the first step, the temperature was raised to 180 °C for 10 min and then it was retained at that temperature for 15 min during the second step. After digestion and cooling, each solution was diluted to a final volume of 10 mL with deionised water.

Instruments and Reagents

An atomic absorption spectrophotometer (AA280Z, Varian) equipped with graphite furnace (GTA 120) and an autosampler (PSD 120) was used for the quantification of Pb, Cd and Cr. As and Hg were quantified through hydride generation-atomic absorption spectrophotometry (HG-AAS) and cold vapour-atomic absorption spectrophotometry (CV-AAS) techniques, respectively using a Varian AA240FS atomic absorption spectrophotometer equipped with a hydride vapour generator (VGA 77). Analytical conditions of AAS employed for the measurements of heavy metals in aqueous solution of fish samples are summarised in Table 1.

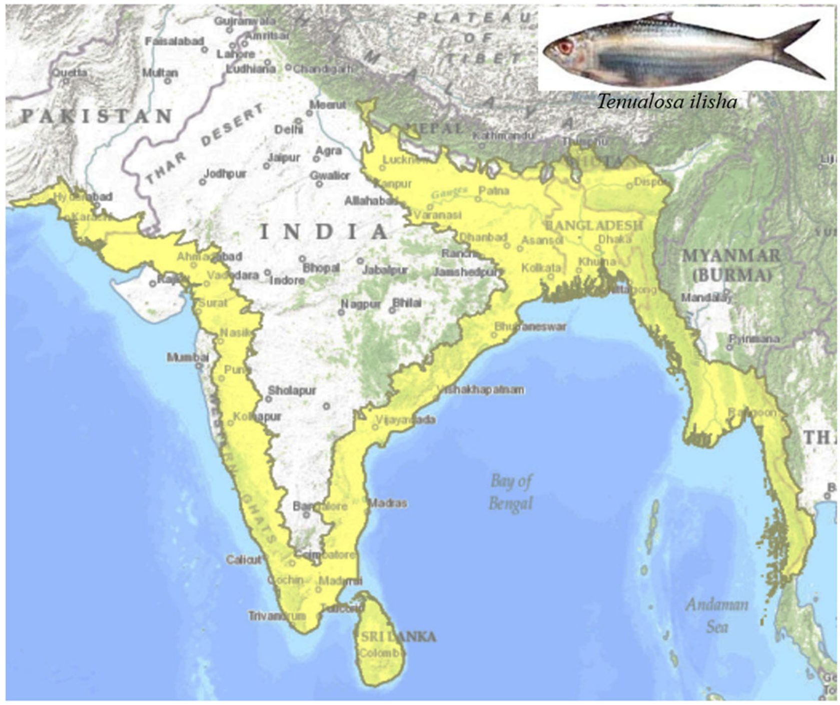

Fig. 1 Distribution of *Tenualosa ilisha* species (inset indicates the camera image of *Tenualosa ilisha*)

The purity of argon and acetylene gases was 99.99%. For the quantification of Pb, Cd and Cr atomic signals were measured in peak area mood and that for As and Hg the atomic signals were measured in integration mood. The working calibration standard solutions were daily prepared by appropriate dilution of the stock standard solutions of 1000 mg L^{-1} using 1% (*w*/*w*) *suprapur* grade nitric acid (Merck, Darmstadt, Germany). Phosphoric acid (Merck, Darmstadt, Germany) was used as a chemical modifier for the determination of Pb, and ammonium phosphate monobasic (Merck, Darmstadt, Germany) for the determination of Cd. Sodium hydroxide (BDH), sodium borohydride (Acros Organics, USA) and hydrochloric acid (Merck, Germany) were used as reductant for the determination As and Hg. Moreover, ascorbic acid (Merck, Germany) and potassium iodide (Merck, Germany) were used as reductant during the sample preparation for As analysis. The reagents used during the sample preparation and analysis were of analytical grade and all solutions were prepared using deionised water with resistivity > 18 MΩ/cm

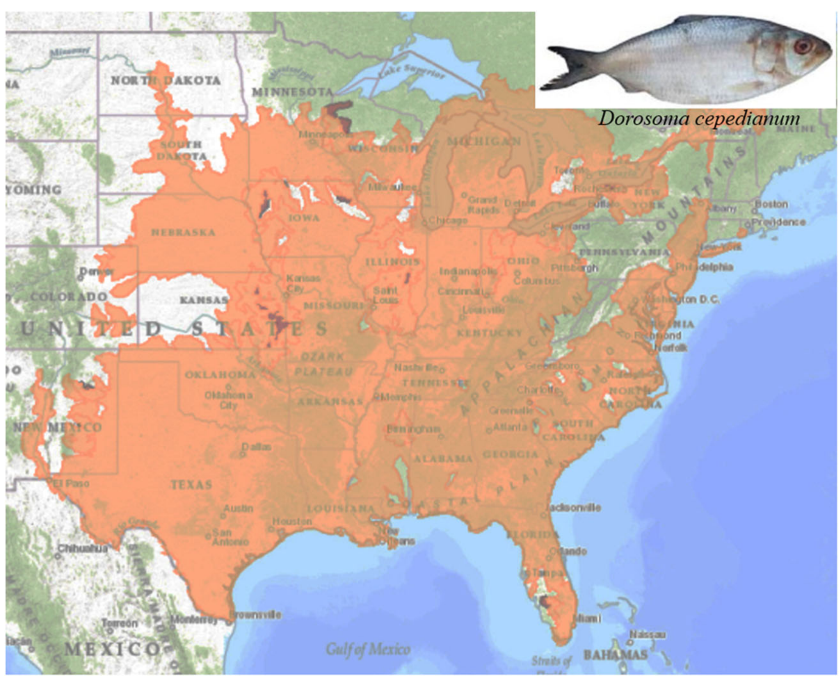

Fig. 2 Distribution of *Dorosoma cepedianum* species (inset indicates the camera image of *Dorosoma cepedianum*)

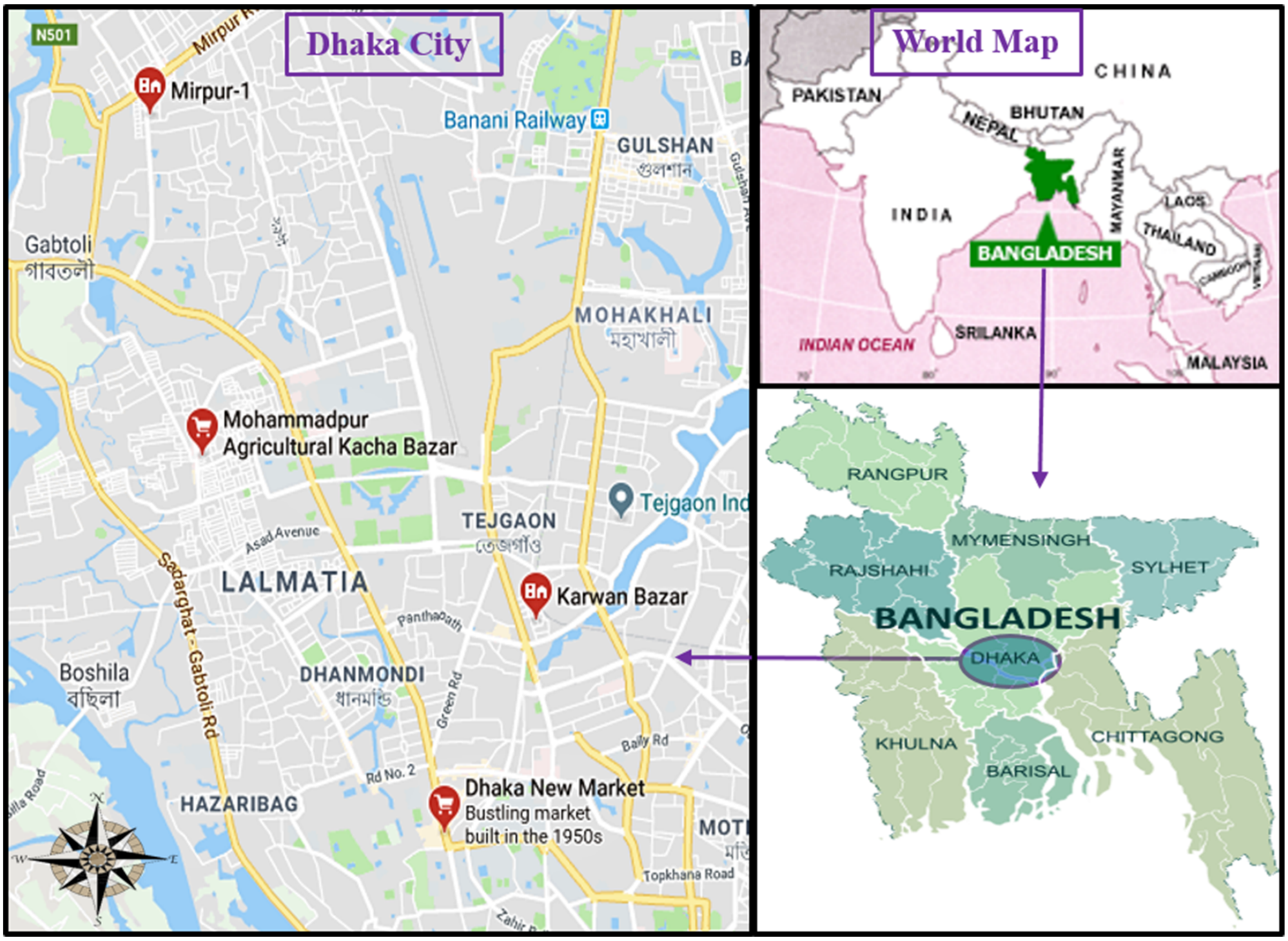

Fig. 3 Map of the sampling area, Dhaka, Bangladesh

produced using an E-pure system (Thermo Scientific, USA). All glassware and containers were cleaned by soaking into 20% nitric acid for at least 24 h and rinsed three times with deionised water prior to use.

Quality Control and Quality Assurance Programs

Internal Quality Control Program

Internal quality control charts (IQCs) are constructed for different heavy metals with the values of their respective quality control standards in order to check the accuracy of the analytical data obtained daily in the laboratory. If no more than 5% of the estimated values exceed the warning limits and none of them exceeds the action or control limit of the IQCs, then the analytical system is considered to be under control [22].

External Quality Control Program

The external quality assurance program is maintained through the participation in proficiency testing organised by LGC, UK and Asia Pacific Metrology Program-Asia Pacific Laboratory Accreditation Cooperation (APMP- APLAC). The results of proficiency tests were within ± 2 *Z*-scores. In order to maintain the laboratory accreditation, it is required to participate in the proficiency testing routinely.

Accuracy of the Method

The accuracy of the analytical technique was checked by analysing a certified reference material NIST CRM 1566a (Oyster Tissue) for the heavy metals, namely, Pb, Cd, Cr, As and Hg, by the same procedure used for fish samples. The mean recoveries of the analysed heavy metals were found to be in between 95.5 and 98.3% and summarised in Table 2. The recovery results indicate the fitness of the method for analysis of heavy metals in fish samples [23].

Statistical Analysis

Multivariate statistical analysis PCA and Pearson's correlation matrix was performed using origin software 9.0 (Origin lab USA) versions. Significance level of Pearson's correlation

Table 1 Analytical conditions of AAS employed for the quantification of heavy metals in aqueous solution of fish samples

Heavy metals	Wavelength (nm)	Slit (nm)	Lamp current (mA)	Instrument quantification limit (mg/kg)	Method quantification limit (mg/kg)	Calibration range (μg/L)
Pb	283.3	0.5	10	0.006	0.06	15–75
Cd	228.8	0.5	4	0.001	0.01	1–6
Cr	357.9	0.2	7	0.005	0.05	4–20
As	193.7	0.5	10	0.0016	0.08	1–10
Hg	253.7	0.5	4	0.0004	0.02	2.5–20

coefficient matrix was considered at two-tailed test at the level of 0.05%.

Calculations

Estimated Daily Intakes

The estimated daily intakes (EDI) for the analysed metals were calculated by using the formula [24]:

$$\mathrm{EDI} = \frac{\mathrm{EFr} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{MC}}{\mathrm{BW} \times \mathrm{TA}} \times 10^{-3}$$

where EFr is the exposure frequency (365 days/year), ED is the exposure duration (70 Years), FIR is the fish ingestion rate (g/person/day) which is 61 g defined for European population [2]; MC is the mean concentrations of metals in fish samples (mg/kg, wet-weight basis); BW is the average body weight (adult 70 kg); TA is the averaging time for non-carcinogens (365 days × number of exposure years, assuming 70 years).

Non-Carcinogenic Risk

Target hazard quotient (THQ) is typically used in order to estimate the potential non-carcinogenic risk of pollutants. It is the ratio of EDI and reference dose (RfD, mg/kg-BW/day) [15] that is expressed by:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}}$$

The *RFDs* are based on 0.004, 0.001, 1.5, 0.003 and 0.0005 mg/kg-BW/day for Pb, Cd, Cr, As and Hg respectively [25]. If the THQ value is lower than 1, the exposed population should not experience any adverse health hazard. Conversely, if the THQ value is 1, there is a potential health risk and the probability of health risk will be increased with increasing THQ value [26].

It has been reported that exposure to more than one pollutant may result in additive and/or interactive effects on human health [27]. Thus, in the present study, cumulative health risk for the investigated heavy metals was assessed by summing the THQ value measured for individual heavy metal and expressed as total THQ (TTHQ) as follows [28]

$$T\mathrm{THQ} = \mathrm{THQ}(\text{toxicant } 1) + \mathrm{THQ}(\text{toxicant } 2) + \ldots \mathrm{THQ}(\text{toxicant } n).$$

The greater the value of TTHQ, the greater the level of concern.

Carcinogenic Risk

The incremental probability of developing cancer in an individual over a lifetime exposure to a potential carcinogen is generally expressed as carcinogenic risk (CR). Cancer risks due to the lifetime exposure to Pb, Cd, Cr and As through the consumption of the selected fish species were measured using the equation [15]:

$$\mathrm{CR} = \mathrm{CSF} \times \mathrm{EDI}$$

where CSF is the carcinogenic slope factor of 0.0085, 0.38, 0.5 and 1.5 mg kg^{-1} day^{-1} for Pb, Cd, Cr and As respectively set by USEPA, USDOE, FAO/WHO [29–31]. EDI is the

Table 2 Heavy metal concentrations (mean ± SD) in certified reference material (NIST CRM 1566a, Oyster Tissue)

Heavy metal	Certified value (mg/kg)	Found value (mg/kg)	Mean recovery (%)
Pb	0.371 ± 0.014	0.361 ± 0.011	97.3
Cd	4.15 ± 0.38	4.06 ± 0.05	97.8
Cr	1.43 ± 0.46	1.39 ± 0.04	97.2
As	14.0 ± 1.2	13.37 ± 0.58	95.5
Hg	0.0642 ± 0.0067	0.0631 ± 0.0027	98.3

estimated daily intake of heavy metals. Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000).

Results and Discussion

Concentrations of Heavy Metals in Fish Muscles

Table 3 represents concentrations of heavy metals Pb, Cd, Cr, As and Hg in the muscle of *Tenualosa ilisha* and *Dorosoma cepedianum* fish species. The metal concentrations are reported as wet-weight basis. The concentrations of the heavy metals in the selected two species of fish were measured as 0.725–1.631 mg kg^{-1} for Pb, 0.020–1.092 mg kg^{-1} for Cd, 0.066–0.787 mg kg^{-1} for Cr, 0.374–1.001 mg kg^{-1} for As and < 0.02 mg kg^{-1} for Hg. The ranking order of mean concentrations of heavy metals was found to be Pb (1.342 mg kg^{-1} > As (0.715 mg kg^{-1}) > Cd (0.462 mg kg^{-1}) > Cr (0.176 mg kg^{-1}) > Hg (< 0.02 mg kg^{-1}).

The mean concentrations of Pb in *Tenualosa ilisha* and *Dorosoma cepedianum* were measured as 1.308 and 1.376 mg/kg respectively. Pb content in *Tenualosa ilisha* was reported in the range of 0.038–0.273 mg/kg from the coast of the Persian Gulf in Iran [4], 0.690 mg/kg from Keti Bunder in Arabian sea, Sindh, Pakistan [32], 2.232–3.175 mg/kg from lower Gangetic delta [33], 0.463 mg/kg from the lower Gangetic delta and coastal West Bengal [34], 0.168 ± 0.01 mg/kg from the north of Persian Gulf [35], 0.003 mg/kg from Shatt Al-Arab River [36], 0.161 mg/kg from Iraqi marine water [37] and 0.035–1.2 mg/kg from Paira River, Bangladesh [38]. There is no or limited value was found for Pb in *Dorosoma cepedianum*. The maximum legislative value of Pb set by the Commission Regulation (EC) No. 1881 [39] is 0.30 mg kg^{-1}. Pb content in *Tenualosa ilisha* and *Dorosoma cepedianum* measured in the present study is above the proposed acceptable limit for human consumption.

The average contents of Cd measured in *Tenualosa ilisha* and *Dorosoma cepedianum* were found to be 0.891 and 0.033 mg/kg respectively. Muscle of *Tenualosa ilisha* shows 27 times higher Cd concentration than that detected in the muscle in *Dorosoma cepedianum*. Cd concentration in *Tenualosa ilisha* in the literature was reported in the range of 0.125–1.77 μg/kg from the coast of the Persian Gulf in Iran [4], 0.030 mg/kg from Keti Bunder in Arabian sea, Sindh, Pakistan [32], 0.01 mg/kg from the lower Gangetic delta and coastal West Bengal [34], 0.02 ± 0.00 mg/kg from the north of Persian Gulf [35], 0.598 mg/kg from Shatt Al-Arab River [36], 0.023 mg/kg from Iraqi marine water ([37]) and 0.001–0.60 mg/kg from Paira River, Bangladesh [38]. There is no or limited information is reported for Cd concentration in *Dorosoma cepedianum*. The amount of cadmium measured in the *Tenualosa ilisha* is higher than the standard of 0.05 mg kg^{-1} set by European Union [39]. However, Cd concentration measured in *Dorosoma cepedianum* is lower than the legislative value, but long period of Cd accumulation in fish may pose health hazards.

In *Tenualosa ilisha* and *Dorosoma cepedianum*, the average concentrations of Cr were found to be 0.094 and 0.258 mg/kg respectively. The Cr concentrations in the selected two species indicate that muscle of *Tenualosa ilisha* shows almost 3 times lower Cr concentration than in the muscle in *Dorosoma cepedianum*. Islam and Habibullah-Al-Mamun [38] reported Cr content as 0.21–0.84 in *Tenualosa ilisha* from Paira River, Bangladesh. From our obtained values, it is found that Cr content in *Tenualosa ilisha* and *Dorosoma cepedianum* fish species is below the legislative value of 1.00 mg kg^{-1} set by MAFF [40].

As is considered as one of the most hazardous heavy metals present in the environment which originates from both natural and anthropogenic activities [41]. It is reported that about 90% of total human exposure of As originates from fish and other seafood [42]. The mean concentrations of As in *Tenualosa ilisha* and *Dorosoma cepedianum* fish species were estimated as 0.679 and 0.751 mg/kg respectively. As content in

Table 3 According to the area, heavy metals concentrations (mean ± SD) in fish species

Sample location	Fish species (local name)	Trace element concentrations (mg/kg)				
		Pb	Cd	Cr	As	Hg
Karwan Bazar	*Tenualosa ilisha* (Jhatka)	1.391 ± 0.278	0.863 ± 0.086	0.168 ± 0.025	1.001 ± 0.051	< 0.02
	Dorosoma cepedianum (Gizzard Shad)	1.447 ± 0.289	0.020 ± 0.002	0.122 ± 0.018	0.429 ± 0.021	< 0.02
Mohammadpur	*Tenualosa ilisha* (Jhatka)	1.484 ± 0.297	0.909 ± 0.091	0.068 ± 0.005	0.768 ± 0.036	< 0.02
	Dorosoma cepedianum (Gizzard Shad)	1.539 ± 0.308	0.050 ± 0.005	0.066 ± 0.007	0.143 ± 0.007	< 0.02
Newmarket	*Tenualosa ilisha* (Jhatka)	1.631 ± 0.326	1.092 ± 0.109	0.114 ± 0.017	0.374 ± 0.014	< 0.02
	Dorosoma cepedianum (Gizzard Shad)	1.559 ± 0.312	0.025 ± 0.003	0.068 ± 0.010	1.158 ± 0.067	< 0.02
Mirpur-1	*Tenualosa ilisha* (Jhatka)	0.725 ± 0.145	0.699 ± 0.069	0.073 ± 0.006	0.573 ± 0.032	< 0.02
	Dorosoma cepedianum (Gizzard Shad)	0.959 ± 0.192	0.036 ± 0.004	0.787 ± 0.118	0.951 ± 0.053	< 0.02

Tenualosa ilisha were reported by earlier studies as 0.001 from Keti Bunder in Arabian sea, Sindh, Pakistan [32] and 0.28–0.83 mg/kg from Paira River, Bangladesh [38]. There is no maximum permissible limit of As in fish sample set by EU, but according to Bangladeshi standard [43], the maximum permissible limit of arsenic is 5.00 mg kg^{-1}. The concentration of As in the two selected fish species is below the Bangladeshi standard.

Hg is a toxic heavy metal and cannot be excreted easily. In the present study, Hg was found in the selected two fish species *Tenualosa ilisha* and *Dorosoma cepedianum* below the detection limit of 0.02 mg/kg. The European Commission Regulation as well as Bangladesh Government Act states permitted Hg concentration of 0.50 mg kg^{-1} [39, 43].

Principal component analysis (PCA) is a tool which can simplify the interpretation of large number of data by reducing the total number of variables to a few factors including the highest variation in the original sets [44]. In the PCA matrix, neighbour distant variables corresponds positive correlation, orthogonal distance variables are not correlated to each other and variables on opposite side of the centre represents significant negative correlation [45]. In Fig. 4, PCA provided two factors considering combined datasets with eigenvalue > 1, by which approximately 68% (PC1 variance of 43.61% and PC2 variance of 24.59%) variability of total data can be explained (Table 4). Figure 4 shows PC where, Cr represents for PC1 and As, Cd and Pb for PC2, respectively. Figure 4 clearly shows that As and Cr are positively correlated, whereas, both of them are negatively correlated with Pb. In addition, there is no correlation observed with As and Cd. Table 5 represents correlation matrix between the heavy metals present in the fish samples. A significant positive correlation is found between As and Cr ($r = 0.34$), whereas, Pb shows strong negative correlation with Cr ($r = -0.45$) as well as lower negative correlation with As ($r = -0.15$) and a slight positive correlation with Cd ($r = 0.05$). However, Cd shows a significant negative correlation with Cr (− 0.31) and As (− 0.03) as well.

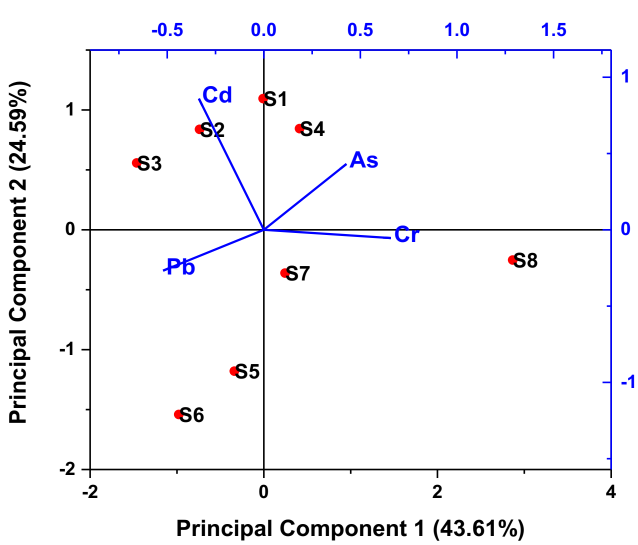

Fig. 4 PCA biplot based on correlation matrix

Table 4 Extracted eigenvectors of coefficient of principal component analysis (PCA)

	Coefficients of PC1	Coefficients of PC2
Pb	− 0.52116	− 0.26799
Cd	− 0.3362	0.85973
Cr	0.65745	− 0.05364
As	0.42793	0.43148

The mechanism behind the As accumulation in the ground water is generally the adsorption of As derivatives ($H_2AsO_4^-$, $HAsO_4^{2-}$, and H_3AsO_3) on hydrous oxide and their co precipitation as well [46]. On the other hand, most important natural source of chromium entry into the water body is leaching from topsoil and rocks. Improper disposal of chromate-processing facilities in to the landfills can be another source of ground water as well as ocean water contamination, where chromium residence time might be several years. Cd is an important metal of earth crust and found small quantity in water, air and soil as well. It is not mined but a by-product of other metal such as Cu, Zn and Pb. Wastewater discharged from metal processing, painting and battery industries is a vital source of Cd and Pb accumulation on surface water [47]. Accumulation of heavy metal into the fish body depended on their food habit, e.g. herbivores fishes can accumulate high content of heavy metals than carnivores [48]. Our studied fishes are mostly ocean cultured and source of the metals in fish are ocean water and phytoplankton as dietary supplements. Though, heavy metal content in fish body is not above the permissible limit, but it is a matter of concern to assess the risk of daily uptake of few amount of heavy metal through dietary supplements.

Table 5 Pearson correlation matrix among four heavy metals (Pb, Cd, Cr, As)

	Pb	Cd	Cr	As
Pb	1			
Cd	0.0561	1		
Cr	− 0.45824*	− 0.31259*	1	
As	− 0.15867*	− 0.03638	0.34024*	1

*Significance of correlation was calculated at 0.05%

Table 6 Comparison of the estimated daily intake of heavy metals from fish species studied with the recommended daily dietary allowances

Metal	Mean concentration (mg/kg-wet wt.)	EDI (mg/day/person)	Recommended daily dietary allowance (mg/day/person)	THQ	TTHQ	Carcinogenic risk (CR)
Pb	1.342	1.17E-03	0.21 (JECFA, 2009)	0.2923	2.7720	9.94E-06
Cd	0.462	4.03E-04	0.06 (JECFA, 2009)	0.4026		4.03E-04
Cr	0.176	1.53E-04	0.20 (RDA, 1989)	0.0001		1.53E-04
As	0.715	6.23E-04	0.13 (JECFA, 2009)	2.0769		6.23E-04
Hg	< 0.02	BDL	0.03 (JECFA, 2009)	BDL		BDL

Estimated Daily Intake

The EDI of Pb, Cd, Cr, As and Hg through the consumption of *Tenualosa ilisha* and *Dorosoma cepedianum* fish species was calculated by considering a 70-kg adult consumes 61 g per day reported for European population and summarised in Table 6. The results depict that Cr contributes the lowest and Pb contributes the highest dietary intake through the consumption of the two selected fish species, which agreed well with the earlier reports [24]. It is found that the values of EDI calculated for the five heavy metals are below the recommended values suggesting no risk to people's health associated with the intake of the studied heavy metals through the consumption of the selected fish species.

Non-Carcinogenic Risk

Target hazard quotient (THQs) of Pb, Cd, Cr, As and Hg due to the consumption of *Tenualosa ilisha* and *Dorosoma cepedianum* fish species was calculated for European adults and summarised in Table 6. The calculated values of THQs are showing the descending order of As (74.93%) > Cd (14.52) > Pb (10.55) > Cr (0.01). THQs for Pb, Cd, Cr and Hg are below 1 indicating no risk to people due to the intake of these metals through the consumption of the selected two species, whereas, THQ value for As exceeds the value 1 indicating potential risk to human for the consumption of selected two species. Moreover, the TTHQ value is calculated as higher than 1 indicating the peoples are at risk due to the intake of the four investigated heavy metals through the consumption of the two fish species.

Carcinogenic Risk

The CR values of Pb, Cd, Cr and As due to exposure from the consumption of *Tenualosa ilisha* and *Dorosoma cepedianum* fish species were calculated as 9.94×10^{-6}, 4.03×10^{-4}, 1.53×10^{-4} and 6.23×10^{-4} respectively. The CR estimated for As is in good agreement with the value reported for marine fish [21]. Generally, the values of CR lower than 10^{-6} are considered as negligible, above 10^{-4} are considered to be unacceptable and lying in between 10^{-6} and 10^{-4} are considered as acceptable range [25, 49]. In the present study, CR for Cd, Cr and As are higher than the unacceptable range indicating the risk of cancer due to exposure to Cd, Cr and As through fish consumption was of concern.

Conclusion

Heavy metals are non-biodegradable and their long-time exposure causes potential human health risk. In the present study, Pb, Cd, Cr, As and Hg were determined in two selected varieties of fish species *Tenualosa ilisha* and *Dorosoma cepedianum* consumed by different countries in the world. The human health risk was assessed from the estimated dietary intake, target hazard quotient and carcinogenic risk. The EDI and THQ values suggest that no risk to human is due to the consumption of the two fish species. However, THQ for As and TTHQ values indicates people are at risk due to the exposure of the targeted heavy metals. In contrast, the carcinogenic risk of Cd, Cr and As due to the consumption of fish was also of concern since the carcinogenic rate in fish was above the acceptable risk level of 10^{-4}.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest to declare.

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